Supporting Information for:

Tumor-Derived Extracellular Vesicles Breach the Intact Blood Brain Barrier *via* Transcytosis

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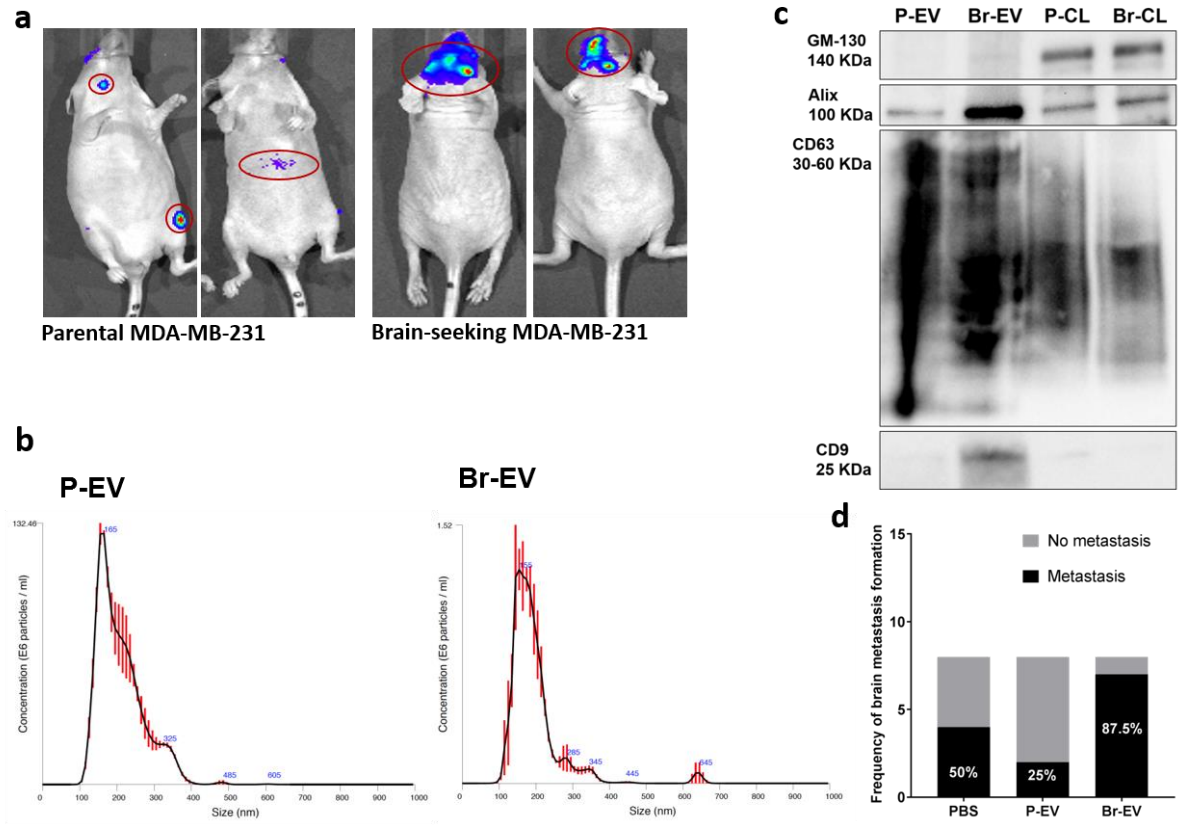
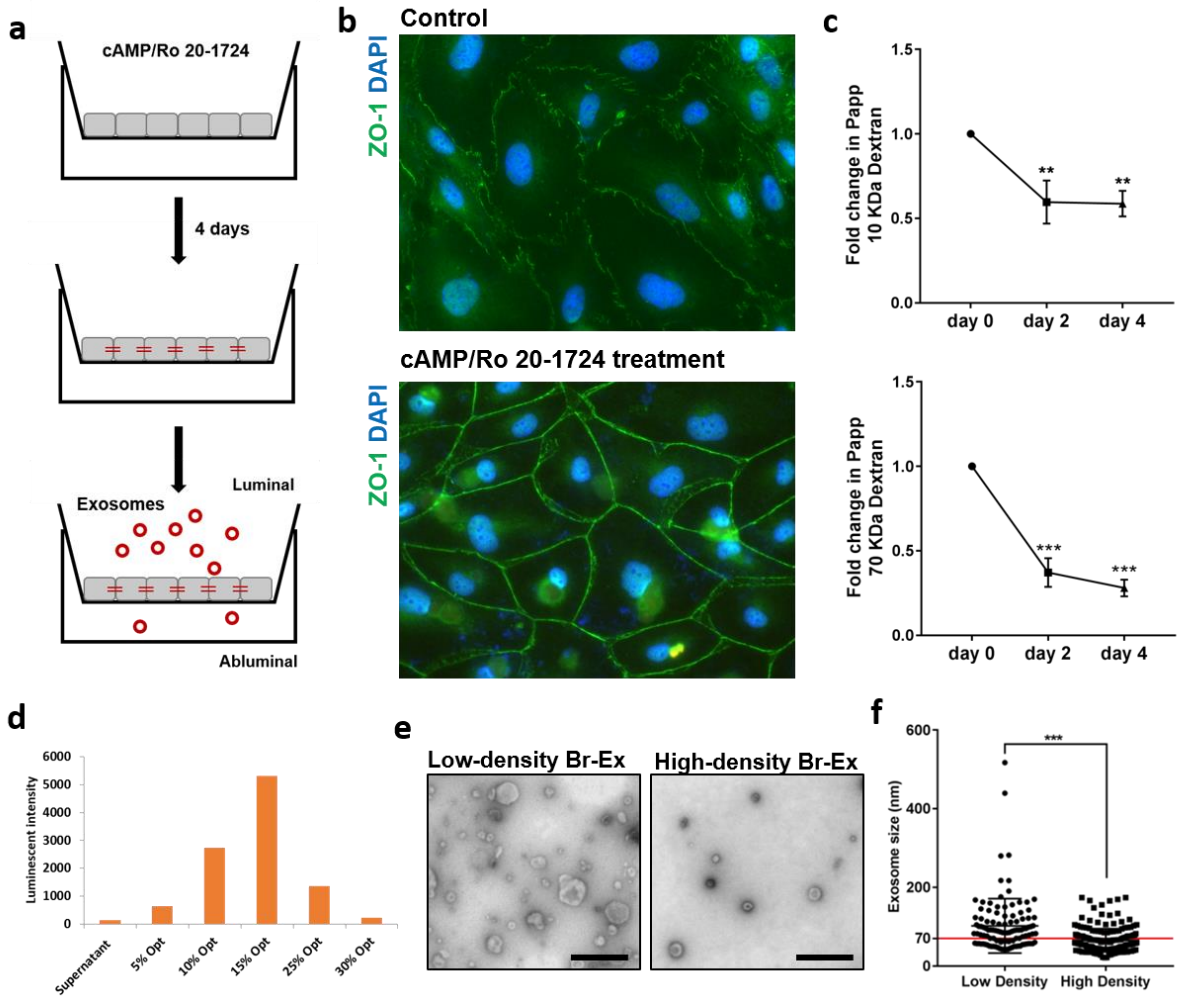
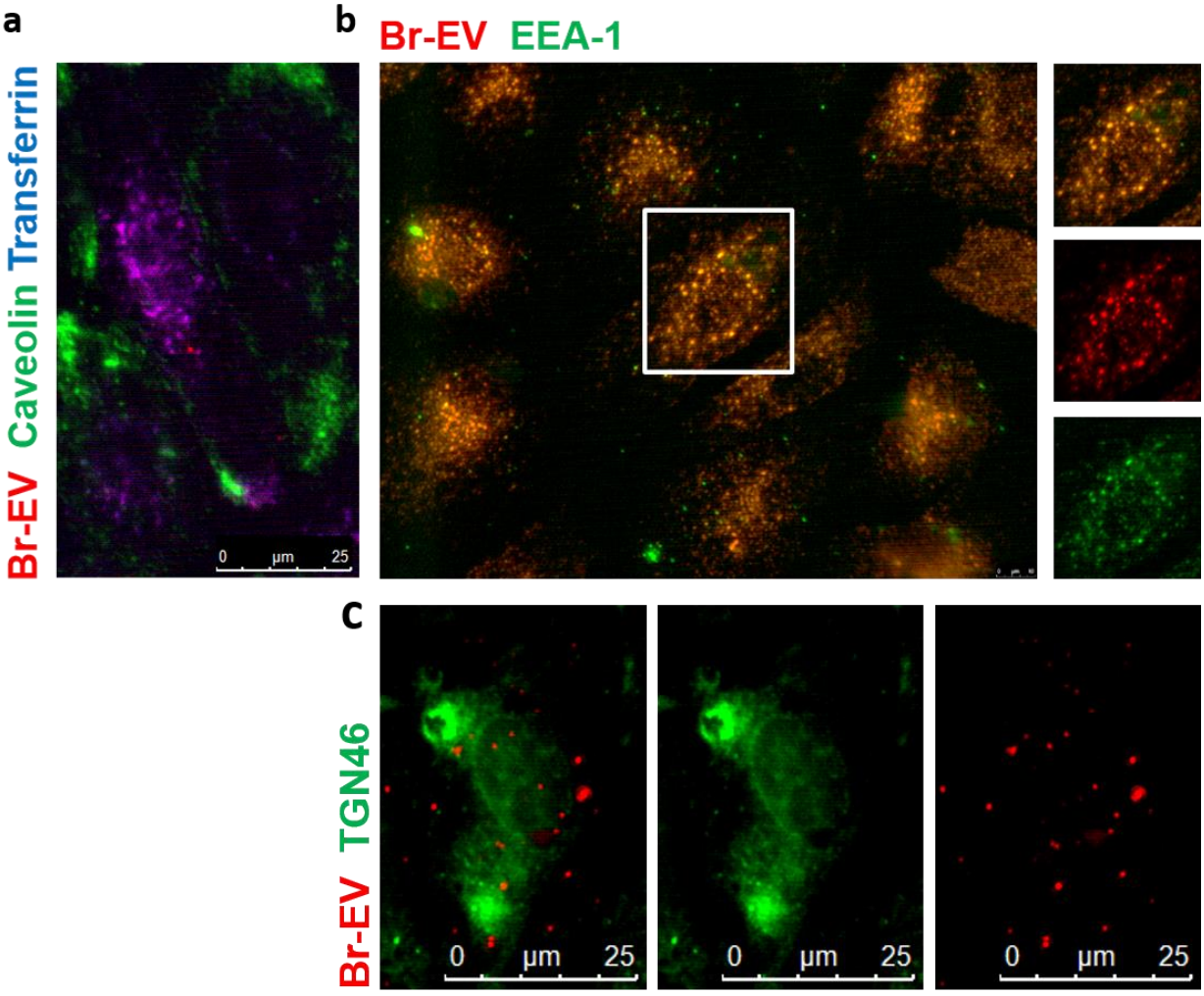
Supplementary Figures



Supplementary Figure S1. Characterization of the cell lines and the isolated EVs. (a) *in vivo* luminescent imaging of metastases following intracardiac injection of parental and brain-seeking MDA-MB-231 cells. (b) Nanoparticle tracking analysis of the size of P-EVs and Br-EVs. (c) Representative western blot images of EV markers CD9, CD63, Alix, and the golgi marker, GM130. (d) The percentage of the mice that developed brain metastases following treatment with PBS, P-EVs, and Br-EVs (n=7 mice per group).



Supplementary Figure S2. Characterization of the *in vitro* BBB model and the transcytosed EVs. (a) Schematic showing static BBB model preparation and transcytosis experiments. (b) Representative images of brain endothelial cells immunostained with anti-ZO-1 antibody following treatment with cAMP and Ro 20-1724 (3 independent experiments). (c) Fold change in permeability coefficient of brain endothelial monolayer to 10 KDa (upper graph) and 70 KDa (lower graph) dextran following treatment with cAMP and Ro 20-1724 (mean ± SD; 3 independent experiments). Statistical analysis was performed using one-way ANOVA with Tukey's correction for multiple comparisons. (d) Luminescent intensity of in density fractions following density gradient fractionation of luciferase-labeled Br-EVs. (e) Electron microscopy images and (F) quantification of the size of EVs isolated from the low density (15% Optiprep) and high density (25% Optiprep) fractions. Statistical analysis was performed using Student's t-test. In all panels, ns, not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.



Supplementary Figure S3. Colocalization of Br-EVs with caveolin, eea1, and TGN46. (a) Representative fluorescence microscopy image of brain endothelial cells immunostained with anti-caveolin 1 antibody from 3 independent experiments. Scale bar, 25 µm. (b) Representative fluorescence microscopy image of brain endothelial cells immunostained with anti-eea1 antibody from 3 independent experiments. Right panels show the magnification of the area in the white square. Scale bar, 25 µm. (c) Representative fluorescence microscopy image of brain endothelial cells immunostained with anti-TGN46 antibody from 3 independent experiments. Scale bar, 25 µm.